II. REMARKS

Formal Matters

Claims 1, 2, 4-9, 15, and 17-23 are pending after entry of the amendments set forth herein.

Claims 1-9, 15, and 17-19 were examined and were rejected.

Claims 1, 4, and 6 are amended. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as acquiescence to any objection or rejection of any claim. Support for the amendments to claim 1 is found in the claims as originally filed, and throughout the specification, in particular at the following exemplary locations: paragraphs 0040 and 0041. Accordingly, no new matter is added by the amendments to claim 1. No new matter is added by the amendments to claims 4 and 6.

Claim 3 is canceled without prejudice to renewal, without intent to acquiesce to any rejection, and without intent to surrender any subject matter encompassed by the canceled claim. Applicants expressly reserve the right to pursue any canceled subject matter in one or more continuation and/or divisional applications.

Claims 20-23 are added. Support for new claims 20-23 is found in the claims as originally filed, and throughout the specification, including the following exemplary locations: claim 20: paragraph 0042; claim 21: paragraph 0049; claim 22: paragraph 0039; and claim 23: paragraph 0047. Accordingly, no new matter is added by these new claims.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Rejection under 35 U.S.C. §112, first paragraph

Claims 1-9, 15, and 17-19 were rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants respectfully traverse the rejection.

A) The Office Action asserted that the state of the prior art appears to recognize a high degree of unpredictability in the field of arginine chemical derivatization.

1) The Office Action stated that α-dicarbonyl compounds bind to cysteine, lysine, and anything hydrophobic; and stated that such "non-specific, indiscriminate, unpredictable α-dicarbonyl activity necessarily affects α-dicarbonyl activity with respect to SDMA and arginine." Office Action, page 3.

The Office Action cited Baburaj et al. ((1994) *Biochim. Biophys. Acta* 1199:253; "Baburaj") to support its assertion of unpredictability.

However, as Applicants have previously explained, the possibility that an α-dicarbonyl compound might modify a cysteine or a lysine residue is irrelevant. Any side reactions that would modify cysteines or lysines would not be expected to adversely affect modification step (a) or detection step (b). Indeed, Baburaj characterizes possible reactions with Cys and Lys as "a rare eventuality that can be ignored." Baburaj, page 262,

column 2, second paragraph. The Declaration of Ken Y. Lin, provided herewith as Exhibit 1, explains that the possibility that an α-dicarbonyl compound might modify a cysteine or a lysine residue in a component of the sample would not be expected to adversely affect the reaction of the α-dicarbonyl compound with guanidino nitrogens of SDMA and arginine that may be present in a biological sample.

2) The Office Action stated that α-dicarbonyl compounds react with arginines to produce multiple arginine derivatives; and stated that such "arginine derivatives necessarily affect α-dicarbonyl activity with respect to SDMA and/or arginine." Office Action, page 4.

The Office Action cited Schwarzenbolz et al. ((1997) *Z. Lebensm. Unters. Forsch. A* 205:121-124; "Schwarzenbolz") and Sopio and Lederer ((1995) *Z. Lebensm. Unters. Forsch.* 201:381-386; "Sopio") to support its position.

However, as Applicants have previously explained, many chemical reactions will produce, in addition to a main product, one or more minor side products. The side products discussed in Schwarzenbolz and in Sopio are minor. Any side products that may be produced in such low quantities would not be expected to adversely affect modification step (b) or detection step (c) as recited in claim 1. The Declaration of Ken Y Lin discusses this aspect.

3) The Office Action stated that α-dicarbonyl compounds react with ADMA; and stated that such ADMA derivatives "necessarily affect α-dicarbonyl activity with respect to SDMA and/or arginine." Office Action, page 4.

The Office Action cited Cooper and Meister ((1978) *J. Biol. Chem.* 253:5407; "Cooper") to support its position. The Office Action stated that Cooper shows that α-dicarbonyl compounds can react with arginyl ε-nitrogen, and that arginyl η-nitrogens are not required for guanidino reactivity with α-dicarbonyl compounds.

However, as explained in the Declaration of Ken Y. Lin, the reaction discussed in Cooper represents a rare derivation of arginine and citrulline, and any quantitative contribution to the method as claimed would be minimal at best.

Furthermore, claim 1 is amended to recite contacting the biological sample with an agent that protects the α-amino group of ADMA and the α-amino group of the at least one of SDMA and arginine. Modification of the α-amino group essentially locks the carbonyl group of the amino acid. Once this happens, the molecule cannot undergo cyclization or alpha-keto acid formation because such would require an intact carbonyl group.

B) The Office Action stated that Applicants' specification provide minimal direction and no working examples successfully performing the claimed two-step method.

The specification provides ample description of reacting a sample with an α-dicarbonyl compound.

The instant specification provides ample description of experimental conditions for performing the claimed method, involving contacting a sample with an α-dicarbonyl compound, to produce modified ADMA and modified arginine, and detecting ADMA.

- The specification provides a list of suitable α-dicarbonyl compounds in paragraphs 0030 and 0031.
- The specification describes suitable concentrations of the α-dicarbonyl compound. Specification, paragraph 0034.
- The specification provides reaction times and temperatures for the reaction between the α-dicarbonyl compound and the SDMA and/or arginine. Specification, paragraphs 0035 and 0038.
- Exemplary reaction conditions are also described. Specification, paragraph 0099.

The Office Action stated that the specification does not clearly describe procedural details for α-dicarbonyl derivatization. However, the specification states:

> An α-dicarbonyl compound is contacted with a biological sample. Generally, the α-dicarbonyl compound is prepared in water, and the pH of the solution is adjusted to 9.0 with 1M NaOH. The α-dicarbonyl compound is generally in a 10X stock solution in a concentration of from about 1 mM to about 500 mM, e.g., from about 1 mM to about 10 mM, from about 10 mM to about 50 mM, from about 50 mM to about 100 mM, from about 100 mM to about 200 mM, from about 200 mM to about 300 mM, from about 300 mM to about 400 mM, or from about 400 mM to about 500 mM. In some embodiments, the α-dicarbonyl compound is in a 10X stock solution in a concentration of from about 50 mM to about 100 mM. A solution containing the α-dicarbonyl compound is added to the biological sample in such a way that the stock solution is diluted 10-fold.
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> and
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> The biological sample is contacted with the α-dicarbonyl compound, and the reaction is allowed to proceed for a period of time of from about 15 seconds to 2 hours, e.g., from about 15 seconds to about 60 seconds, from about 1 minute to about 15 minutes, from about 15 minutes to about 30 minutes, from about 30 minutes to about 60 minutes, or

> from about 1 hour to about 2 hours. In a particular embodiment, the reaction is allowed to proceed for 1 hour to about 2 hours in the dark at room temperature (e.g., at about 22 °C).

Specification, paragraphs 0034 and 0035. The description in paragraphs 0034 and 0035 is sufficient for those skilled in the art to carry out the reaction of a biological sample with an α-dicarbonyl compound.

Furthermore, the specification provides exemplary reaction conditions. The specification states:

> This product is relatively stable at acidic pH's, but at pH>10, the reaction was observed to be reversible. Typically, phenylglyoxal is dissolved in water and the pH is adjusted to 9.0 with 1M NaOH. A solution containing phenylglyoxal is then added to the sample in such a way that the stock solution is diluted 10-fold. The reaction proceeds in the dark at room temperature for 60-180 minutes (Reference: Tawfik DS, Walter JM, Modification of arginine side chains with *p*-hydroxyphenylglyoxal, *The Proteins Protocol Handbook 2002,* 2nd edition, Humana Press Inc.)

Specification, paragraph 0099.

Thus, given the guidance in the specification, those skilled in the art could readily carry out a method as claimed.

The specification provides ample description of methods for detecting ADMA.

The Office Action stated that the specification does not describe a detecting means capable of detecting ADMA. As discussed during the telephone interview, the specification describes **at least four** methods of detecting ADMA. Specification, paragraphs 0043-0067. These methods include HPLC, capillary electrophoresis, immunoassays, and liquid chromatography-tandem mass spectrometry.

- **HPLC** As discussed in the specification, HPLC methods for detecting ADMA were known in the art. Specification, paragraph 0044. The instant specification provides a detailed description of an exemplary method of using HPLC to detect ADMA in a sample. Specification, paragraphs 0045-0050. See, e.g., Teerlink et al. (2002) *Anal. Biochem.* 303:131-137; Dobashi et al. (2002) *Analyst* 127:54-59; Pi et al. (2000) *J. Chromatogr. B. Biomed. Sci. Appl.* 742:199-203; Chen et al. (1997) *J. Chromatogr. B. Biomed. Sci. Appl.* 692:467-471; Anderstam et al. (1997) *J. Am. Soc. Nephrol.* 8:1487-1442; and Pettersson et al. (1997) *J. Chromatogr. B. Biomed. Sci. Appl.* 692:257-262.

- **Capillary electrophoresis** As discussed in the specification, capillary electrophoresis methods of detecting ADMA were known in the art. Specification, paragraphs 0052-0053. See, e.g., Causse et al. (2000) *J. Chromatogr. B. Biomed. Sci. Appl.* 741:77.
- **Immunoassays** As described in the instant specification, immunoassays can be designed that take advantage of the ability to distinguish modified SDMA and modified arginine from ADMA. Substitute Specification, paragraphs 0054-0065. Design and execution of such immunoassays is well within the skill level of those in the art.
- **Liquid chromatography-tandem mass spectrometry** As discussed in the specification, liquid chromatography-tandem mass spectrometry methods of detecting ADMA were known in the art. Specification, paragraph 0066. See, e.g., Vishwanathan et al. (2000) *J. Chromatogr. B. Biomed. Sci. Appl.* 748:157-166.

The specification provides ample description as to how to modify an α-amino group.

The specification states that an α-dicarbonyl compound may react with the α-amino group of arginine, SDMA, and ADMA, and that, should such a reaction occur, an optional step of derivatizing the α-amino group of arginine, SDMA, and ADMA is performed before the modification of the guanidino nitrogen groups of arginine and SDMA. Many methods are known in the art for modifying (protecting) the α-amino group. Specification, paragraph 0040.

The specification goes on to describe numerous agents that are protecting groups for α-amino groups. Specification, paragraphs 0041 and 0042. Such compounds were known in the art as of the priority date of the instant application. Furthermore, conditions for reacting an α-amino group with such compounds were well known as of the priority date of the instant application.

The Office Action has not provided sufficient reasoning to doubt the enablement of the instant specification.

The specification asserts that SDMA and arginine present in a biological sample can be reacted with an α-dicarbonyl compound such that the modified SDMA and modified arginine so generated are distinguishable from ADMA, which is not modified by the α-dicarbonyl compound. A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. §112, first paragraph, unless there is a reason to doubt the objective

truth of the statements contained therein which must be relied on for enabling support.[1] The Office Action has not provided sufficient reason as to why a person skilled in the art would doubt that the method as claimed is enabled by the instant specification. As discussed above, and in previous responses to Office Actions, the art cited by the Office does not support a conclusion that the instant claims are not supported by the specification.

The Office Action stated that "no working examples successfully performing the claimed two-step method" are provided in the specification. However, it is well established that compliance with the enablement requirement under 35 U.S.C. §112, first paragraph, does not require or mandate that a specific example be disclosed. The specification need not contain a working example if the invention is otherwise disclosed in such a manner that one skilled in the art would be able to practice the invention without undue experimentation.[2] Furthermore, "Nothing more than objective enablement is required, and therefore it is irrelevant whether [a] teaching is provided through broad terminology or illustrative examples."[3]

As discussed above, the instant specification provides ample description such that those skilled in the art could readily practice the claimed method without undue experimentation. As such, the instant claims are in compliance with the enablement requirement of 35 U.S.C. §112, first paragraph.

Conclusion as to the rejection under 35 U.S.C. §112, first paragraph

Applicants submit that the rejection of claims 1-9, 15, and 17-19 under 35 U.S.C. §112, first paragraph, has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejection under 35 U.S.C. §102(b)

Claims 1, 3, and 17 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Ogawa et al. ((1987) *Arch. Biochem. Biophys.* 252:526 ("Ogawa").

The Office Action stated that Ogawa describes a method of detecting ADMA, comprising: a) contacting a sample with an α-dicarbonyl compound, wherein the sample comprises ADMA, SDMA, and arginine; and b) detecting ADMA. The Office Action stated that Ogawa describes α-dicarbonyl compounds that inherently modify guanidino nitrogens. Applicants respectfully traverse the rejection.

Ogawa discusses metabolic fates of ADMA, and the kinetics of renal clearance of ADMA. Ogawa discusses experiments in which ADMA or SDMA, either radioactively labeled or unlabeled, was injected

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[1] *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).
[2] *In re Borkowski*, 164 USPQ 642,645 (CCPA 1970).

intraperitoneally into rats. Urine, plasma, or tissue samples were collected. The samples were processed, then fractionated on ion exchange columns, and the eluates analyzed. The eluates contained metabolites of ADMA or SDMA.

Ogawa does not disclose or suggest a method that involves contacting a biological sample with an α-dicarbonyl compound. As such, Ogawa cannot anticipate claim 1, or any claim depending directly or indirectly from claim 1.

As discussed above, claim 1 is amended to recite contacting the biological sample with an agent that protects the α-amino group of ADMA and the α-amino group of the at least one of SDMA and arginine. Ogawa neither discloses nor suggests a method that involves contacting a biological sample with an agent that protects the α-amino group of ADMA and the α-amino group of the at least one of SDMA and arginine, followed by reacting the sample with an α-dicarbonyl compound. As such Ogawa cannot anticipate claim 1, or any claim depending directly or indirectly from claim 1.

Conclusion as to the rejection under 35 U.S.C. §102(b)

Applicants submit that the rejection of claims 1, 3, and 17 under 35 U.S.C. §102(b) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Withdrawn rejection

The Office Action stated that the previous rejection of claim 1 under 35 U.S.C. §112, second paragraph, has been "principally withdrawn."

[3] *In re Robins* 166 USPQ 552 at 555 (CCPA 1970).

III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-276.

Respectfully submitted,
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Date: March 24, 2008

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